Environment International 139 (2020) 105672

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Ultrasensitive monitoring of DNA damage associated with free radicals exposure using dynamic carbon nanotubes bridged interdigitated electrode array

Hui Zhao[a,b,1], Misha Liu[a,1], Tao Jiang[b], Jinjin Xu[a], Huirong Zhang[a], Chaofan Yu[a], Zipeng Liu[a,c], Ying Wang[a,c,*], Longhua Tang[b,*]

[a] College of Environmental Science and Engineering, State Key Laboratory of Pollution Control and Resource Reuse Study, Tongji University, 1239 Siping Road, Shanghai 200092, China
[b] State Key Laboratory of Modern Optical Instrumentation, College of Optical Science and Engineering; International Research Center for Advanced Photonics, Zhejiang University, Hangzhou 310027, China
[c] Shanghai Institute of Pollution Control and Ecological Security, Shanghai 200092, China

ARTICLE INFO

Handling Editor: Da Chen

Keywords:
DNA
Free radicals
Interdigitated electrode array Biosensor
Carbon nanotube

ABSTRACT

There are currently increasingly concerns over DNA damage related to free radicals due to their vital roles in human health, especially high-performance detection method. Herein, we report an ultra- sensitive monitoring of DNA damage associated with free radicals exposure using interdigitated electrode (IDE) array for the first time. The proposed IDE array was equipped with DNA-wrapped carbon nanotube-based bridges, which utilized the DNA damage mechanism due to the free radicals' attack and the efficient electrical detection nature of the interdigitated electrode. Experiments have been performed, and the results showed the device's capability for detecting DNA damage induced by multiple free radicals generated from different sources, including the Fenton reaction, UV radiation and cigarette smoke, showing the promising ability for DNA damage detection. In addition, the carbon nanotubes bridge-based interdigitated electrode sensor enabled different levels of sensing of DNA damage with great sensitivity and a wide detection range. It was illustrated that the ultrasensitive detection of free radicals generated from ultraviolet radiation (15 min – 125 min), cigarette smoke tar (1 μg/mL to 10 μg/mL) and Fenton reaction under different concentration of H_2O_2 (2.5 pM – 100 pM), have been detected successfully. Typically, the IDE array supports further performance improvement for the electrochemical detection in an ultrasensitive and high throughput route.

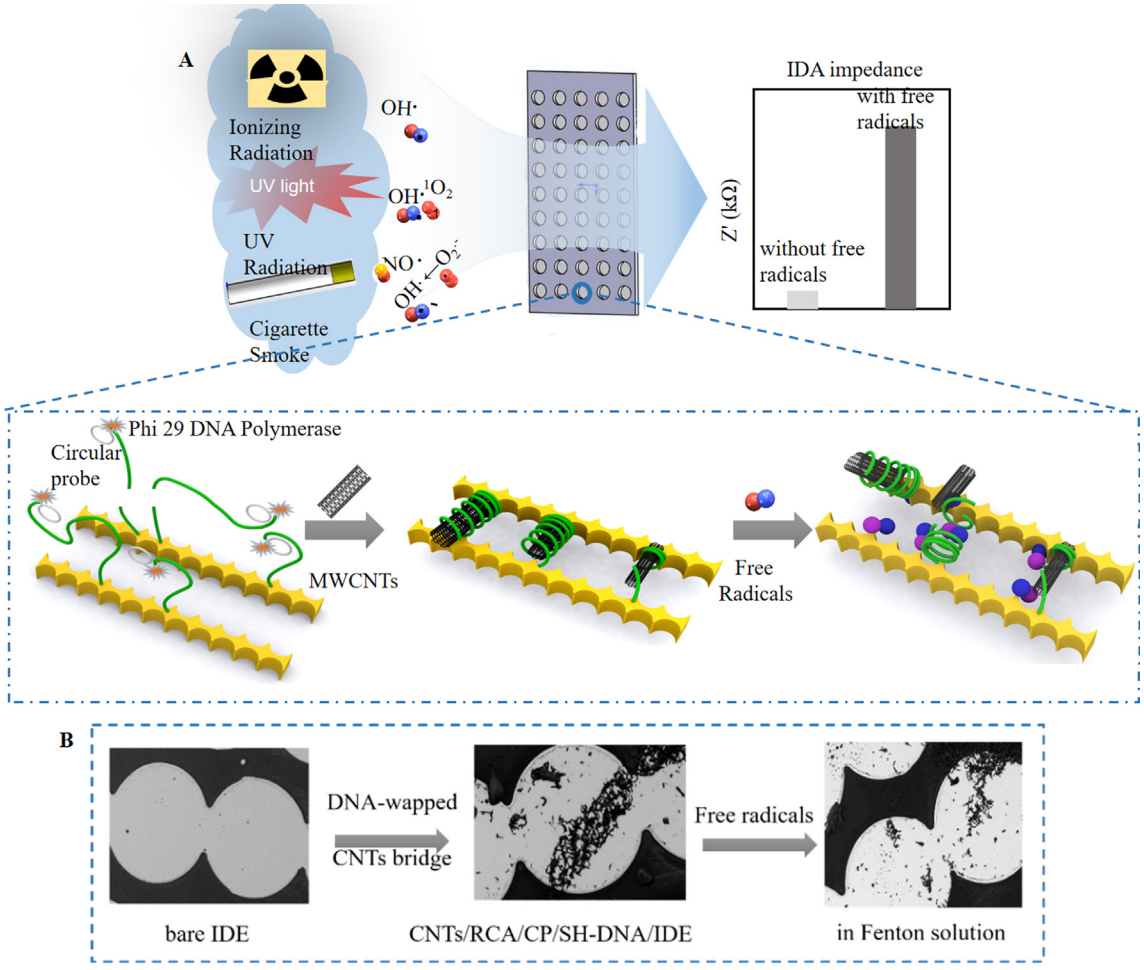
1. Introduction

Deoxyribonucleic acid (DNA) is of great importance in life with their function in the storage, copying and transfer of information. However, life processes or environmental issues (e.g., UV or chemical exposure, medical therapies, smoking, etc.), could generate or induce multiple free radicals, which will introduce damage to DNA through DNA strand breaks, base oxidation or deletion (Norbury and Hickson, 2001; Rouse and Jackson, 2002), leading to various physiological and pathological processes, including atherosclerosis, cancer, diabetes and neurodegenerative diseases (Wang et al., 2017; Liu et al., 2017; Yin et al., 2011). Owing to increasing pollution and radiation, free radicals induced by environmental issues should deserve more attention.

In recent years, some typical sensing strategies for DNA damage monitoring have been proposed, including high performance liquid chromatography analysis (Renner et al., 2000; Tarun et al., 2006); gas chromatography-mass spectrometry analysis (Kudo et al., 2014); fluorescence analysis (Pouget et al., 2000; Viswesh et al., 2010); ^{32}P post-labeling analysis (Spencer et al., 2011); electrochemical method (Chen et al., 2012; Hlavata et al., 2012; Hlavatá, 2014; Fojta, 2016; Iffelsberger et al., 2018; Svitková and Labuda, 2019); and single cell gel electrophoresis analysis (Quincozes-Santos et al., 2007). Despite a variety of methods, the electrochemical method has stood out in this field for its convenience and reliability. However, considering environmentally-induced free radicals' multiplicity (˙OH, $O_2^{\bullet -}$, NO•) and low concentration, it is highly desired to develop new ultrasensitive

* Corresponding authors at: College of Environmental Science and Engineering, State Key Laboratory of Pollution Control and Resource Reuse Study, Tongji University, 1239 Siping Road, Shanghai 200092, China (Y. Wang).
E-mail addresses: yingwang@tongji.edu.cn (Y. Wang), lhtang@zju.edu.cn (L. Tang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.envint.2020.105672
Received 8 November 2019; Received in revised form 16 March 2020; Accepted 17 March 2020



Scheme 1. (A) Schematic illustration of the design of interdigitated electrode (IDE) array with DNA-wrapped carbon nanotubes-based bridges. In the presence of free radicals, the DNA-wrapped nanobridges could control the characteristic current or conductance response of the IDEs. In the presence of free radicals, DNA would be damaged and/or cleaved, thereby inducing the electrochemical signal change of IDEs. (B) SEM images of bare IDE, MWCNTs/RCA/CP (circular probe)/SH-DNA/IDE before and after immersed in Fenton solution.

electrochemical method, particularly one that can work in real conditions (Wang et al., 2019).

We herein propose an ultrasensitive monitoring of DNA damage associated with free radicals exposure, which integrates an interdigitated electrode (IDE) array with DNA wrapped-carbon nanotubes-based bridges (Scheme 1). The nanobridges were designed to be located among the IDEs, which were comprised of the long single-strand DNA produced by rolling circle amplification (RCA) and multi-walled carbon nanotubes (MWCNTs). In the presence of free radicals, the DNA-wrapped nanobridges could control the characteristic current or conductance response of the IDEs because of the DNA damaging effect. DNA would be damaged and/or cleaved by free radicals, thereby inducing the electrochemical signal change of IDEs. With the MWCNTs bridge-based IDEs, DNA damage levels induced by free radicals could be sensitively quantified by setting up a model of quantitative analysis based on the mechanism of Fenton reaction (Valko et al., 2004; Zhang et al., 2010). To improve the detection throughput and avoid any spin-trapping agents or pre-treatment, we further employed an IDE array on the 96-well plate (Fig. S1), which could significantly improve the detection capacity for multiple free radicals from different situations, such as Fenton reaction, UV radiation, and cigarette smoke exposure. The developed methodology based on MWCNTs bridge-based IDEs has been demonstrated with promising ability for simultaneously quantifying DNA damage related to multiple free radicals, providing a universal application for fast and effective detection of DNA damage.

2. Materials and methods

2.1. Materials

DNA sequences (5′-HS-$(CH_2)_6$-AAAAAAGTTGGTAC GTTAGGAACA TCAAACGACAGCCA), T4 DNA ligase, Exonuclease I, Exonuclease III, deoxyribonucleotides mixture (dNTPs), 10 × T4 DNA ligase reaction buffer, were purchased from TaKaRa Biotech Company (Dalian, China) and HPLC purified phi29 DNA polymerase, 10 × phi29 DNA polymerase reaction buffer and BSA were obtained from New England Biolabs (NEB, USA). Multi-walled carbon nanotubes (MWCNTs), were obtained from Aladdin (Shanghai, China). Polyethylene glycol octadecyl ether was purchased from Sigma-Aldrich (USA). Micro Gap Electro-chemical Sensor Array was obtained from ACEA Biosciences Inc. Ethidium bromide (EB) and DNA Marker were obtained from Sangon Biotech (Shanghai, China). All DNAs were diluted with 10 mM PBS Buffer (PBS; pH 7.4; 10 mM phosphate, 0.1 M NaCl) containing 0.1 M NaCl. Washing buffer in the experiment was 10 mM PBS (pH 7.4). All of the solutions were prepared with ultra-pure water. Cigarettes were purchased at local stores in Shanghai, China. Cigarettes were stored at room temperature for up to 3 months and at or below 16 ℃ for longer term storage. Prior to sampling, cigarettes were conditioned with ISO 3402: 1999 recommended conditions of 22 ℃ and 60% relative humidity for at least 48 h, but less than 10 days, with the pack unsealed.

2.2. Samples preparation

Free radical ˙OH from Fenton reaction is generated by Fenton solution (PBS buffer solution containing $FeSO_4$ and H_2O_2 at the molar ratio 1:4 with pH 4.0). Cigarettes were smoked on an automated, rotary 20-port RM20H smoking machine (Borgwaldt, Germany). Cigarettes were smoked following the ISO 3308:2000 regimen. One sample consisted of ten commercially available filtered cigarette smoked sequentially. Cigarette tar was collected on the filter directly and then diluted with acetone at the final concentration of 50 μg/mL, stored at 4 ℃ for further experiments.

2.3. Electrochemical detection of free radical from Fenton reaction

The DNA-wrapped MWCNTs-bridged IDE was immersed in Fenton solution at 37 ℃ for 30 min. The DNA strand was attacked by ˙OH generated by Fenton reaction. Then, the electrode was rinsed with ultra-pure water. Cyclic-Voltammetry (CV) and electrochemical impedance spectra (EIS) were recorded in 0.5 mM $[Fe(CN)_6]^{3-/4-}$ solution. Each measurement was repeated three times.

2.4. Electrochemical detection of free radical from UV radiation

The single-strand DNA-wrapped MWCNTs-bridged IDE was exposed to UV radiation, of which EIS characterization in the 0.5 mM $[Fe(CN)_6]^{3-/4-}$ solution was recorded with exposure time of 15 min, 30 min, 45 min, 70 min, 95 min, 110 min, and 125 min.

2.5. Electrochemical detection of free radical from cigarette tar

EIS characterization in the 0.5 mM $[Fe(CN)_6]^{3-/4-}$ solution of DNA-wrapped MWCNTs-bridged IDE was tested under different concentration of cigarette smoke tar of 1 μg/mL, 3 μg/mL, 5 μg/mL, and 10 μg/mL.

3. Results and discussion

3.1. Fabrication and feasibility of MWCNTs bridged IDE sensor for DNA damage

To prove the design concept of the sensing strategy, the DNA damage sensors were fabricated (Fig. 1A). Firstly, the gold IDE surface was functionalized with thiol-terminated DNA (SH-DNA) via the Au-S bond. Then, by adding a circulate probe (CP) and phi29 DNA polymerase, the solid phase RCA was performed on the IDE, producing super-long DNA strands with hundreds of repeated sequences. The RCA procedure was also verified by a solution-based RCA experiment (Fig. 1B). Next, we introduced MWCNTs to intertwine with the produced long DNA products, thereby realizing the connection of the gaps on the IDE array. MWCNTs are a primary family of carbon nanomaterials, which are characteristically excellent super electrochemical conductors and are highly stability. To improve the solubility and the interaction with the DNA chain, polyethylene glicol octadecyl ether (PEG) was used to disperse and functionalize the MWCNTs. After functionalization with PEG, the MWCNTs showed excellent solubility and stability in aqueous solutions (Fig. S2A). Additionally, the nanotube morphology and its nanostructure have been kept well after with PEG functionalization, as illustrated in the consistent TEM imaging and XRD characterization (Fig. S2C, S2D and S2E in SI).

In order to illustrate the DNA-wrapped MWCNTs-bridged IDE sensor, we first verified that the long chain DNA and MWCNTs can be entangled, and the conformational change of DNA was analyzed by circular dichromatogram (CD). CD is sensitive to changes in the composition of DNA within 200–320 nm. According to previous reports (Yang et al., 2008; Tang et al., 2012), the adsorption and interaction between MWCNTs and single-strand DNA are much stronger than double-strand DNA through π - π packing (Tang et al., 2006). As shown in Fig. 1C, CD curve for long-chain DNA wrapped on MWCNTs was changed obviously within the wavelength ranged from 200 nm to 320 nm. After being intertwined with MWCNTs, peaks corresponding to DNA at 276 nm and 225 nm were reduced, illustrating that the interaction between DNA and MWCNTs occurred.

To demonstrate the feasibility of MWCNTs bridge-based IDE array, we monitored the electrochemical response of IDE sensor. In a two-electrode system, one electrode of the IDE sensor was used as a working electrode and the other one was used as a counter electrode and reference electrode, simultaneously. As shown in Fig. 1D and S3, EIS and CV were collected on different IDE in $[Fe(CN)_6]^{3-/4-}$ solution using $Fe^{2+/3+}$ as the electrochemical probe. In the presence of DNA-wrapped MWCNTs, the IDE possessed an obvious oxidation and reduction peak, which belong to the single electron transfer from $[Fe(CN)_6]^{3-/4-}$, indicating the successful connection in the IDE (Fig. S3, curve e). By contrast, no obvious oxidation or reduction peaks were observed without the nanobridges (Fig. S3, curve b, c and d). Moreover, the corresponding impedance obtained from EIS proved the connection of IDE with the DNA-wrapped MWCNTs (Fig. 1E). As for the gapped IDE, the resistance was around 17 kΩ. After being immobilized with SH-DNA and hybridized with CP, the resistance was increased to 53 kΩ and 61 kΩ, respectively. After the solid-phase RCA was complete, the resistance was decreased to 26 kΩ, which can be ascribed to the weak electronic conductance of the DNA backbone. Whereas in the presence of DNA-wrapped nanobridges on the IDE, the resistance was sharply dropped to 480 Ω. Therefore, the typical electrochemical response and reduced resistance demonstrated the successful construction of DNA-wrapped nanobridge on IDE surface.

3.2. DNA damage monitoring induced by free radicals using MWCNTs bridged IDE sensor

As known, Fenton reaction is an important metal-mediated pathway for the generation of ˙OH from hydrogen peroxide. The ˙OH radicals are able to interact with all the DNA components and damage bases as well as base free sites strand breaks (Zhang et al., 2010). In this paper, we used Fenton chemistry to produce ˙OH, and investigated the practical utility of the nanobridge based IDE array biosensor for DNA damage induced by free radicals. Fenton reaction mechanism is shown by Scheme 1 (Imlay and Linn, 1988):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + (OH)^- + \bullet OH \quad (1)$$

The number of generated ˙OH radicals is dependent on the concentration of Fenton reagents (Qian et al., 2010; Liang and Guo, 2007; Liu et al., 2005). As shown in Fig. S4, Fig. 2A and 2B, with the addition of free radicals onto the DNA-wrapped MWCNTs-bridged IDE, the peak current in CV decreased and the resistance in EIS increased, demonstrating the disassembly of DNA-wrapped nanobridge and thereafter the disconnection of the IDE.

Next, we investigated the specificity of DNA damage to the free radicals using resulted MWCNTs bridged-IDE. In the Fenton reaction system, the coexistence of H_2O_2 and Fe^{2+} are necessary for producing ˙OH. As we can see from Fig. 2A and S4, no obvious changes were obtained when the IDE was immersed in either 400 pM H_2O_2 solution or 200 pM $FeSO_4$ respectively. Importantly, in Fenton solution, free radicals attacked DNA-wrapped MWCNTs bridge-based IDE, causing violent impedance increase (Fig. 2B). To further verify the production of ˙OH generated by Fenton reaction, we carried out the Electron Spin Resonance (ESR) test of the Fenton solution. As shown in the inset of Fig. 2B, there is a very typical ESR curve with quartet splitting for ˙OH. The SEM imaging also illustrated that DNA strain was damaged owing to the free radicals' attack in Fenton solution (Scheme 1B).

Furthermore, we explored the DNA damage detection for the different amounts of free radicals using MWCNTs bridge-based IDE sensor. This was controlled by tuning the concentration of Fe^{2+} or H_2O_2 as

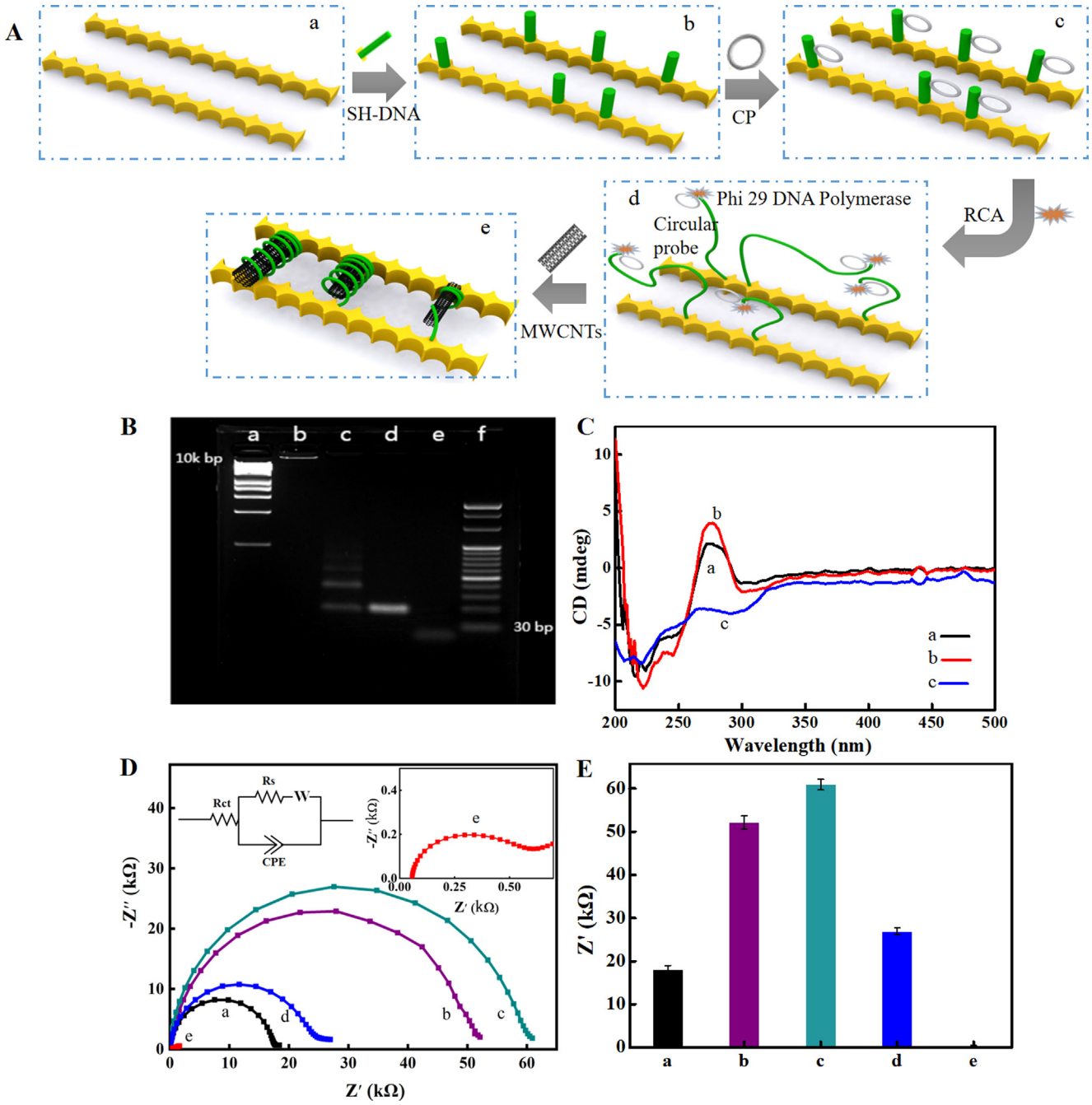


Fig. 1. (A) Scheme of the construction of IDE with MWCNTs based bridges for DNA damage detection. (a) Bare IDE, (b) SH-DNA/IDE, (c) CP/SH-DNA/IDE, (d) RCA/CP/SH-DNA/IDE, and (e) MWCNTs/RCA/CP/SH-DNA/IDE. (B) Electrophoresis image of solution-based RCA, DNA Marker 10 kbp (a), RCA product (b) circular probe ligated by T4 DNA ligase (c), 74-nucleotide-long circular probe DNA (d), 22-nucleotide-long primer DNA (e), DNA Marker 300 bp (f). (C) CD of RCA product (a), MWCNTs wrapped with long strand DNA (b) and the blank PBS buffer solution (c). (D) EIS and (E) impedance of (a) bare IDE, (b) SH-DNA/IDE, (c) CP/SH-DNA/IDE, (d) RCA/CP/SH-DNA/IDE, and (e) MWCNTs/RCA/CP/SH-DNA/IDE in the 0.5 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 M KCl (pH 7.4). Inset of (D) is the equivalent circuit, where Rs/Rct/CPE/W represented solution resistance, charge-transfer resistance, the constant phase element and Warburg impedance, respectively. Rct was electrode impedance; detailed figure of curve e. The bias potential of EIS ranged from 10 kHz to 0.1 Hz at 50 mV.

shown previously (Hájková and Barek, 2017; Huang et al., 2012). To obtain the best detection condition, we carried out the experimental optimization for the concentration of CP, MWCNTs (Fig. S6) and RCA reaction time (Fig. S7). On the basis of experiments results, 1.5 μL CP, 1 μg/mL MWCNTs and 2 h for RCA reaction were selected as the optimized experimental parameters for the following works.

We then investigated EIS and CV responses for free radicals generated from different concentrations of H_2O_2 (Fig. 2C and Fig. S8A). Impedance increased with increasing H_2O_2 concentration (Fig. 2D), and meanwhile CV area decreased (Fig. S8B). Furthermore, a linear relationship between impedance and logarithm of H_2O_2 concentration from 2.5 pM to 100 pM was found at: Z' =3166.9 ln (C) − 4915.01 (R^2 = 0.994, RMS of the slope was 0.083, origin was at 1.609) (inset of Fig. 2D). The detection limit of 1.04 pM was calculated from the regression parameters (3σ).

In addition to using the Fenton reaction to produce the ˙OH and to test the specificity of nanotubes-bridged IDE sensor for DNA damage induced by free radicals, we also explored a proposed sensing strategy to detect free radicals generated from UV radiation at 365 nm and cigarette smoking (Detailed information about UV radiation and cigarette

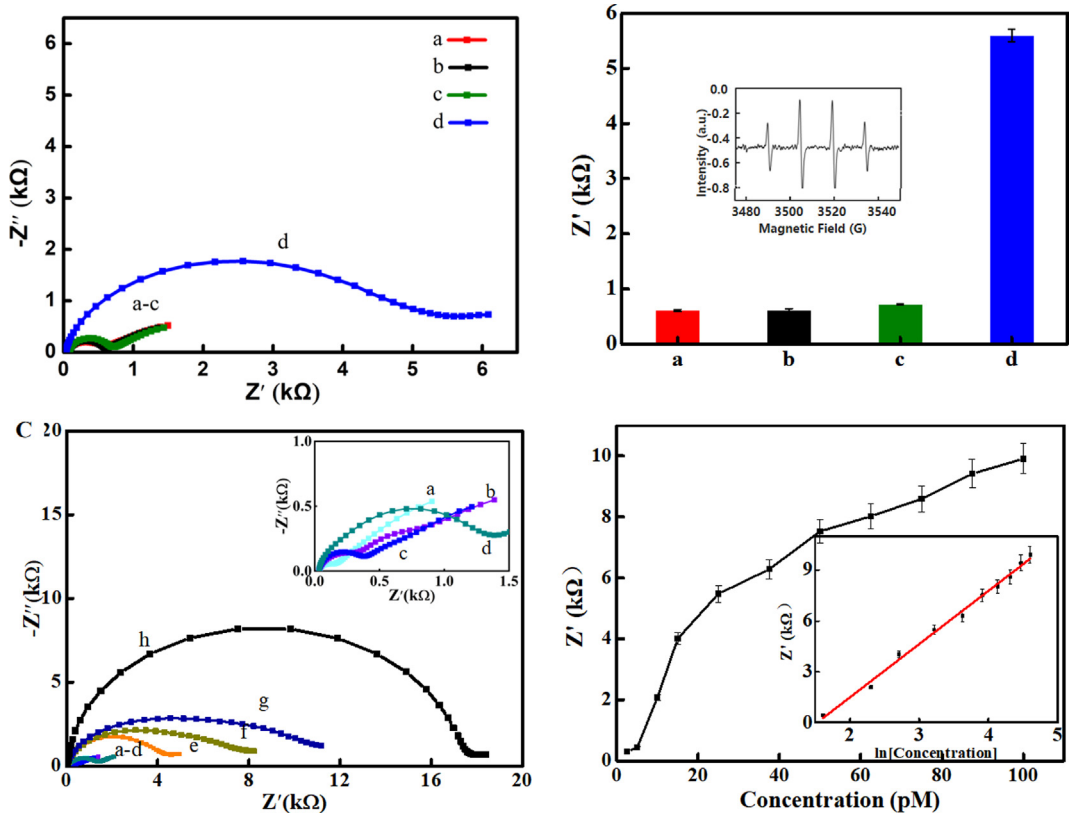


Fig. 2. (A) EIS and (B) impedance of (a) MWCNTs/RCA/CP/SH-DNA/IDE, MWCNTs/RCA/CP/SH-DNA/IDE immersed in PBS buffer solution containing (b) 400 pM H_2O_2 solution, (c) 200 pM $FeSO_4$ solution, and (d) Fenton solution (400 pM H_2O_2 and 200 pM $FeSO_4$) for 30 min (pH 4.0). Inset of (B) is ESR spectra of $^{\cdot}OH$. The bias potential of EIS ranged from 10 kHz to 0.1 Hz at 50 mV. (C) EIS of (a) DNA-wrapped-MWCNTs bridged IDE in Fenton solution with H_2O_2 concentration at (b) 10 pM, (c) 20 pM, (d) 40 pM, (e) 100 pM, (f) 200 pM, (g) 400 pM and (h) bare electrode. (D) The relationship of DNA-wrapped MWCNTs-bridged IDE sensor between impedance and H_2O_2 concentration. And inset of (D) was relationship of DNA-wrapped $MWCNT_S$-bridged IDE sensor between impedance and logarithm of H_2O_2 concentration. The bias potential of EIS ranged from 10 kHz to 0.1 Hz at 50 mV.

tar collection are shown in the SI). As shown in Fig. 3A, IDE array was exposed to UV radiation and EIS and CV responses increased with exposure time from 15 min to 125 min (Fig. 3B and S9A), the linear relationship between impedance of MWCNTs bridge-based IDE array corresponding to DNA damage and exposure time was obtained (Fig. 3C). At the same time, CV area decreased linearly from 15 min to 125 min of UV exposure time (Fig. S9B). With Free radicals generated from cigarette smoking are considered as one of the environmental free radicals, which are very harmful to human health (Chouchane et al., 2006; Maskos et al., 2005). The WHO has proved that free radicals in cigarette tar are of a very high concentration (Wang et al., 2016). Cigarettes were smoked on an automated, rotary 20-port RM20H smoking machine (Fig. 3D). Cigarette tar was collected on the filter directly and then diluted with acetone for a final concentration from 1 μg/mL to 10 μg/mL for experiments. As shown in Fig. 3E and F, with the addition of the diluted cigarette tar into the sensing well, DNA damage intensified, nanotubes-bridged IDE sensor could successfully monitor the damage levels related to concentration change of cigarette smoke tar, suggesting its capability for the detection of DNA damage induced by free radicals in cigarette smoke.

4. Conclusions

In summary, we have demonstrated an ultrasensitive DNA-wrapped MWCNTs bridge-based IDE sensor for the first time, by using an interdigitated electrode array based 96-well plate, leading to the capability of DNA damage detection. Free radicals from different sources including the Fenton reaction, UV radiation and cigarette smoke tar could be sensed effectively. The developed DNA-wrapped carbon nanotube-based bridges provided the sensitive responses for electrochemical detection with nano-effects. Further, sensor's responses vary as the concentration changes, which reveals the ability to monitor the amount of free radicals. Thus, this study has illustrated an efficient DNA damage sensor suitable for multiple forms of free radicals detection from various sources, which may also enable accurate quantitative measurement in the future. We could further improve the performance for the electrochemical detection, to enable the detection of free radicals in an ultrasensitive and high throughput way.

CRediT authorship contribution statement

Hui Zhao: Methodology, Writing - review & editing, Data curation. **Misha Liu:** Data curation, Writing - original draft. **Tao Jiang:** Visualization, Investigation. **Jinjin Xu:** Validation, Formal analysis. **Huirong Zhang:** Software. **Chaofan Yu:** Validation, Writing - review & editing. **Zipeng Liu:** Resources, Visualization. **Ying Wang:** Supervision, Funding acquisition, Conceptualization. **Longhua Tang:** Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the National Science Foundation of

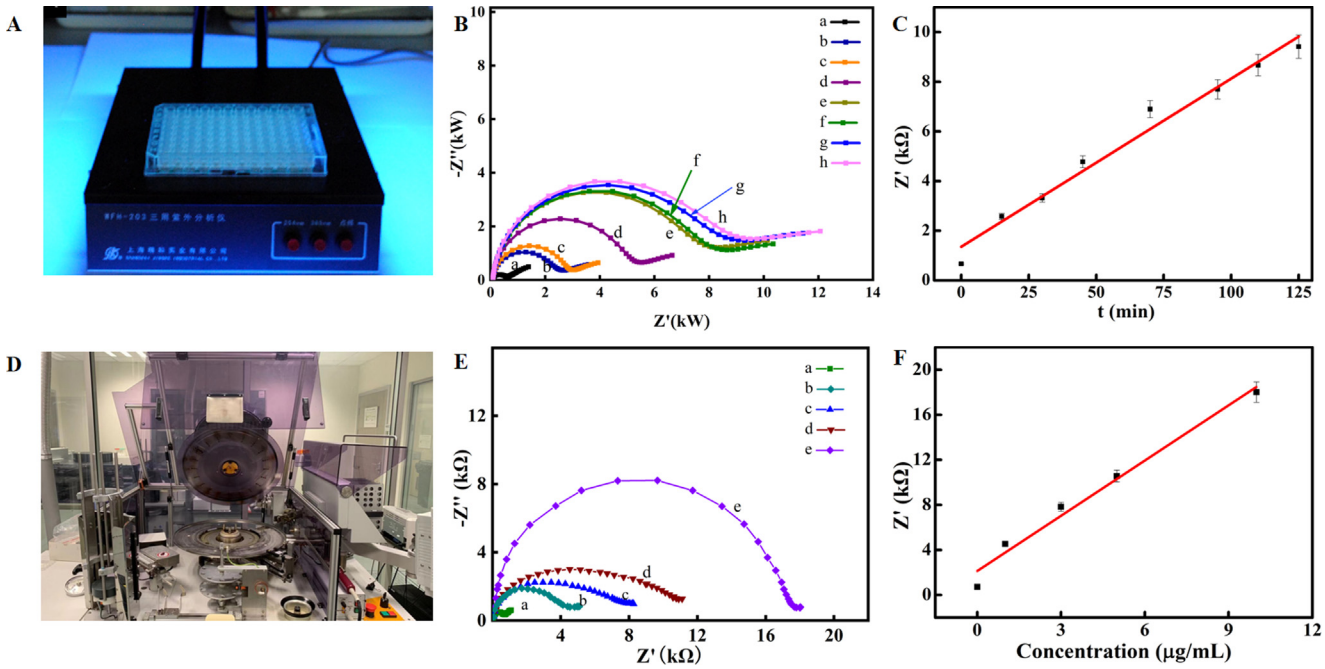


Fig. 3. (A) Photo of the MWCNTs bridge-based IDE array exposed to ultraviolet radiation. (B) EIS characterization in the 0.5 mM $[Fe(CN)_6]^{3-/4-}$ solution of MWCNTs bridge-based IDE array exposed at different times of ultraviolet radiation, (a) to (h) 0 min, 15 min, 30 min, 45 min, 70 min, 95 min, 110 min, 125 min. The bias potential of EIS ranged from 10 kHz to 0.1 Hz at 50 mV. (C) The relationship of MWCNTs bridge-based IDE sensor between resistance and UV exposure time. (D) Photo of cigarette smoking machine. (E) EIS characterization in the 0.5 mM $[Fe(CN)_6]^{3-/4-}$ solution of MWCNTs/RCA/CP/HS-DNA/IDE in different concentrations of cigarette smoke, (a) to (e) 0 μg/mL, 1 μg/mL, 3 μg/mL, 5 μg/mL and 10 μg/mL. (F) The relationship of MWCNTs bridge-based IDE sensor between resistance and concentrations of cigarette smoke. The bias potential of EIS ranged from 10 kHz to 0.1 Hz at 50 mV.

China (NSFC, No. 21305046), the National Program for Support of Top-Notch Young Professionals, Shanghai Rising-Star Program (18QA1404300), the Fundamental Research Funds for the Central Universities (22120180102), and Young Excellent Talents in Tongji University (2015KJ001), Science Technology Commission of Shanghai Municipality (18DZ1204400).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105672.

References

Norbury, C.J., Hickson, I.D., 2001. Cellular responses to DNA damage. Annu. Rev. Pharmacol. Toxicol. 41, 367–401.
Rouse, J., Jackson, S.P., 2002. Interfaces between the detection, signaling naling, and repair of DNA damage. Science 297, 547–551.
Wang, X., Gao, F., Zhang, X., 2017. Initiator-loaded gold nanocages as a light-induced free-radical generator for cancer therapy. Angew. Chem. Int. Ed. 56, 9029–9033.
Liu, M., Xu, J., Yang, F., Gu, Y., Chen, H., Wang, Y., Li, F., 2017. Sensitive electrochemical detection of DNA damage based on in situ double strand growth via hybridization chain reaction. Anal. Bioanal. Chem. 409, 6821–6829.
Yin, H., Xu, L., Porter, N.A., 2011. Free radical lipid peroxidation: mechanisms and analysis. Chem. Rev. 111, 5944–5972.
Renner, Thomas, Fechner, Thomas, Scherer, Gerhard, 2000. Fast quantification of the urinary marker of oxidative stress 8-hydroxy-2′-deoxyguanosine using solid-phase extraction and high-performance liquid chromatography with triple-stage quadrupole mass detection. J. Chromatogr. B Biomed. Sci. Appl. 738 (2), 311–317. https://doi.org/10.1016/S0378-4347(99)00542-3.
Tarun, Maricar, Bajrami, Besnik, Rusling, James F., 2006. Genotoxicity screening using biocatalyst/DNA films and capillary LC−MS/MS. Anal. Chem. 78 (2), 624–627. https://doi.org/10.1021/ac0517996.
Kudo, K.I., Ito, H., Ihara, S., Terato, H., 2014. Quantitative analysis of oxidative DNA damage induced by high-voltage pulsed discharge with cavitation. J. Electrostat. 73, 131–139.
Pouget, J.P., Douki, T., Richard, M.J., Cadet, J., 2000. DNA damage induced in cells by and UVA radiation as measured by HPLC/GC-MS and HPLC-EC and comet assay. Chem. Res. Toxicol. 13, 541–549.
Viswesh, Velliyur, Gates, Kent, Sun, Daekyu, 2010. Characterization of DNA damage induced by a natural product antitumor antibiotic leinamycin in human cancer cells. Chem. Res. Toxicol. 23 (1), 99–107. https://doi.org/10.1021/tx900301r.
Spencer, Wendy A., Jeyabalan, Jeyaprakash, Kichambre, Sunita, Gupta, Ramesh C., 2011. Oxidatively generated DNA damage after Cu(II) catalysis of dopamine and related catecholamine neurotransmitters and neurotoxins: Role of reactive oxygen species. Free Radical Biol. Med. 50 (1), 139–147. https://doi.org/10.1016/j.freeradbiomed.2010.10.693.
Chen, Yang, Xiong, Huayu, Zhang, Xiuhua, Wang, Shengfu, 2012. Electrochemical detection of in situ DNA damage induced by enzyme-catalyzed Fenton reaction. Part I: in phosphate buffer solution. Microchim Acta 178 (1-2), 37–43. https://doi.org/10.1007/s00604-012-0808-3.
Hlavata, L., Benikova, K., Vyskocil, V., Labuda, J., 2012. Evaluation of damage to DNA induced by UV-C radiation and chemical agents using electrochemical biosensor based on low molecular weight DNA and screen-printed carbon electrode. Electrochim. Acta 71, 134–139.
Hlavatá, L., Vysko il, V., Beníková, K., Borbélyová, M., Labuda, J., 2014. DNA-based biosensors with external Nafion and chitosan membranes for the evaluation of the antioxidant activity of beer, coffee, and tea. Open Chem. 12, 604–611.
Fojta, M., Da"hel, A., Havran, L., Vysko il, V., 2016. Recent progress in electrochemical sensors and assays for DNA damage and repair. TrAC Trends Analyt. Chem. 79, 160–167.
Iffelsberger, C., Raith, T., Vatsyayan, P., Vysko il, V., Matysik, F.M., 2018. Detection and imaging of reactive oxygen species associated with the electrochemical oxygen evolution by hydrodynamic scanning electrochemical microscopy. Electrochem. Acta 281, 494–501.
Svitková, V., Labuda, J., Vysko il, V., 2019. Batch injection analysis with amperometric detection for DNA biosensing applications. Electroanalysis 31, 2001–2006.
Quincozes-Santos, A., Andreazza, A.C., Nardin, P., Funchal, C., Gottfried, C., 2007. Resveratrol attenuates oxidative-induced DNA damage in c6 glioma cells. Neurotoxicology 28, 886–891.
Wang, Z., Yi, K., Lin, Q., Yang, L., Chen, X., Chen, H., Liu, Y., Wei, D., 2019. Free radical sensors based on inner-cutting graphene field-effect transistors. Nat. Commun. 10, 1544.
Valko, M., Izakovic, M., Mazur, M., Rhodes, C.J., Telser, J., 2004. Role of oxygen radicals in DNA damage and cancer incidence. Mol. Cell. Biochem. 266, 37–56.
Zhang, Q., Qiao, Y., Hao, F., Zhang, L., Wu, S., Li, Y., Li, J., Song, X., 2010. Fabrication of a biocompatible and conductive platform based on a single-stranded DNA/graphene nanocomposite for direct electrochemistry and electrocatalysis. Chem.-A European J. 16, 8133–8139.
Yang, R., Tang, Z., Yan, J., Kang, H., Kim, Y., Zhu, Z., Tan, W., 2008. Noncovalent assembly of carbon nanotubes and single-stranded DNA: an effective sensing platform for probing biomolecular interactions. Anal. Chem. 80, 7408–7413.
Tang, L., Chang, H., Liu, Y., Li, J., 2012. Duplex DNA/graphene oxide biointerface: from fundamental understanding to specific enzymatic effects. Adv. Funct. Mater. 22, 3083–3088.
Tang, X., Bansaruntip, S., Nakayama, N., Yenilmez, E., Chang, Y., Wang, Q., 2006. Carbon

nanotube DNA sensor and sensing mechanism. Nano Lett. 1632–1636.
Imlay, J.A., Linn, S., 1988. DNA damage and oxygen radical toxicity. Science 240, 1302–1309.
Qian, P., Ai, S., Yin, H., Li, J., 2010. Evaluation of DNA damage and antioxidant capacity of sericin by a DNA electrochemical biosensor based on dendrimer-encapsulated Au-Pd/Chitosan composite. Microchim. Acta 168, 347–354.
Liang, M., Guo, L., 2007. Photoelectrochemical DNA sensor for the rapid detection of DNA damage induced by styrene oxide and the Fenton reaction. Environ. Sci. ™Technol. 41, 658–664.
Liu, J., Roussel, C., Lagger, G., Tacchini, P., Girault, H.H., 2005. Antioxidant sensors based on DNA-modified electrodes. Anal. Chem. 77, 7687–7694.
Hájková, A., Barek, J., Vysko il, V., 2017. Electrochemical DNA biosensor for detection of DNA damage induced by hydroxyl radicals. Bioelectrochemistry 116, 1–9.
Huang, W., Xie, W., Shi, Y., Luo, H., Li, N., 2012. A simple and facile strategy based on Fenton-induced DNA cleavage for fluorescent turn-on detection of hydroxyl radicals and Fe^{2+}. J. Mater. Chem. 22, 1477–1481.
Chouchane, S., Wooten, J.B., Tewes, F.J., Wittig, A., Müller, B.P., Veltel, D., Diekmann, J., 2006. Involvement of semiquinone radicals in the in vitro cytotoxicity of cigarette mainstream smoke. Chem. Res. Toxicol. 19, 1602–1610.
Maskos, Z., Khachatryan, L., Cueto, R., Pryor, W.A., Dellinger, B., 2005. Radicals from the pyrolysis of tobacco. Energy ™fuels 19, 791–799.
Wang, Y., Liu, M., Zhu, Y., Cheng, K., Wu, D., Liu, B., Li, F., 2016. Identifying the tobacco related free radicals by UPCC-QTOF-MS with radical trapping method in mainstream cigarette smoke. Talanta 160, 106–112.

Hui Zhao, She received bachelor's degree from Jilin University in 2015, and began her PhD at Zhejiang University since 2015. Her research interests include electrochemical sensor, nanopore technology, silicon photonic devices.

Misha Liu was born in 1992. She received her Master degree in Environmental Science from Tongji University in 2017. She began her PhD at Tsukuba University in the program of sustainable environmental studies since 2019.

Tao Jiang, He is a Master of Science in optical engineering in Zhejiang University. His research focuses on nanoparticle-based optical inspection, including Raman, photo-acoustic and OCT.

Jinjin Xu received her BS degree from College of Chemistry and Life Sciences at Zhejiang Normal University in 2012. She is currently a post-graduate under the supervision of A.P. Ying Wang at Tongji University. Her major research focuses on the synthesis and application of functionalized carbon nanomaterials.

Huirong Zhang was born in 1997. She received her BS degree in Environmental Engineering in 2018 from the Fujian Normal University (China).She began her MS degree at Tongji University in the Institute of Environmental Science and Technology since 2018.

Chaofan Yu was born in China in 1995. He received his BS degree in Applied Chemistry in 2017 from Tongji University and began his MS degree with professor Ying Wang at Tongji University. His current research interests mainly focus on electrochemical monitoring analysis.

Zipeng Liu was born in China in 1997. He is in his BS degree in Environmental Science at Tongji University and is going to do his MSc in ETH Zurich in 2019. In 2017 he joined Professor Wang's group and started the research about the detection of tracing pollutants using nanomaterials.

Ying Wang was born in Gansu, China in 1984. She received her BS degree in Applied Chemistry in 2007 from the Wuhan University (China). She began her PhD with Professor Jinghong Li at Tsinghua University since 2007. In 2012, she joined Professor Honglai Liu's group at East China University of Science and Technology as a post-doctoral fellow. Since 2015, she has been an associate professor of Tongji University and was employed as a professor in 2019. Her research interests include analysis and detection of environmental pollutants, electrochemical technology and its environmental applications, and application of functional nanomaterials/nanotechnology in environmental pollution control.

Longhua Tang, He received MSc from East China University of Science and Technology in 2008, and then obtained PhD from Tsinghua University in 2013. After that, he moved to Zhejiang University as a researcher associate and was promoted to associated professor in 2016. His research focuses on molecular photonics and electronics, as well as single-molecular techniques.